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Summary, Conclusions, and Commentary on the Molecular Genetics of Childhood Renal Tumors

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When Louise Strong and I analyzed the genetics of Wilms' tumor (WT), we concluded that there was probably one major gene responsible for the disease and that its location would be revealed by the discovery of a deletion associated with aniridia. We now know that *WT1* is that gene. However, to our surprise, *WT1* seems to account for a minor fraction of all cases of WT. Furthermore, our expectation that bilaterally affected cases always signify a germline mutation may be incorrect. Here at this meeting we found that there are some answers to the implied questions, but there are also questions yet unanswered.

Certainly *WT1* has proved to be an interesting gene. It also met our early expectation that such tumor-specific genes should be tissue-specific in their expression, and important in development. This was a considerable relief after the discovery that *RB1* was not only not tissue-specific, but rather ubiquitous, in its expression. In his fine overview of WT, Max Coppes noted the association of the Denys-Drash syndrome with constitutional missense mutations and the WAGR syndrome with constitutional deletions. The beautiful work on the expression of *WT1*, *Pax 2*, and other genes during development, presented by Jordan Kreidberg and Greg Dressler, constitutes the best example of gene expression during human embryogenesis and abnormality thereof in carcinogenesis. The *WT1* knockout mouse informs us that if human heterozygotes for *WT1* mutations did not develop tumors, we would know the gene only for developmental defects in heterozygotes and prenatal lethality in homozygotes.

The association of *WT1* mutations and the WAGR syndrome with intralobar nephrogenic rests immediately suggests that WT1 expression is necessary for the normal differentiation of nephroblasts. However, is heterozygosity for mutation sufficient to produce rests, perhaps by a dominant negative mutation, or is loss of the second copy of *WT1* necessary? Dan Haber has found rests of both types, so we are still left wondering what is necessary to produce an intralobar rest, and what is necessary to convert a rest cell into a tumor cell.

The estimated frequency of WTs with abnormality of *WT1* has risen some, due to more careful surveillance of mutations in the gene. Vicky Huff reported on mutations in exon 1, which are laborious to detect because of its intensely rich GC content. Interestingly, exon 1 mutations are deletions or insertions, suggesting difficulty in replication in a GC-rich region of DNA. Still, it seems unlikely that more than 20% of tumors will reveal such mutations, and some minor part of that fraction will involve a germline mutation or loss. The possible antisense regulation of *WT1* by *WIT1*, discussed by Bryan Williams, suggests that *WT1* could be rendered nonfunctional by mutation of *WIT1*. It may also be that splicing defects that are not associated with mutation in *WT1*, as mentioned by Dan Haber, could impair function of the gene.

About 50% of WTs show loss of heterozygosity (LOH) for chromosome 11p markers, but the fact that some tumors showed LOH at 11p15, but not at 11p13, the site of *WT1*, pointed strongly to another important gene in the region of 11p15. The mapping of the Beckwith-Wiedemann syndrome (BWS) gene to this region reinforced the idea of its importance, since BWS predisposes to WT. The realization that loss of the maternal allele is characteristic of most WTs led to the idea that genomic imprinting at 11p15 was involved. Rosanna Weksberg discussed the different mechanisms that bring about the expression of both alleles of insulin-like growth factor 2 (*IGF2*) in BWS. Some cases show loss of imprinting (LOI) without loss of the maternal chromosome, by activation of the maternal allele. In some instances, genetic recombination and LOI occur postzygotically and can lead to nonheritable BWS. Andy Feinberg found LOI in about 70% of WTs, so this region plays a major role in sporadic WT. That fraction, taken together with cases that uniquely involve *WT1*, suggests that the great majority of WTs involve abnormality at one of these two regions. It would be important to know how many WTs involve neither of these regions.

IGF2 may be operating like an oncogene by perpetuating nephroblasts, and may account for the perilobar rests observed in BWS patients. However, the development of WT in BWS patients occurs at a very low frequency,

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suggesting that another event is essential. Some investigators believe that a critical tumor suppressor gene resides in the 11p15 region, but its detection has proved elusive so far. The paternally imprinted gene *H19* has been considered as a candidate since it shows growth-suppressive effects. In BWS, and in tumors that show LOI for *IGF2,* both copies of *H19* are hypermethylated and are unexpressed, as discussed by Andy Feinberg and Tony Reeve. Ben Tycko described two cases in which by-allelic *H19* hypermethylation was found in normal tissue as well as in the tumor tissue, which must also be the case with BWS and WT. If loss of *H19* function is important in the origin of WT, it is clearly not sufficient to cause WT. In a quest for critical genes at 11p15, Marcel Mannens is finding genetic heterogeneity in BWS cases, although three different cDNA fragments recognized the same RNA transcript. A distinctly separate region of DNA was associated with atypical BWS patients who also had hemihypertrophy (HH).

HH has long been known to predispose to WT, and since it can be associated with BWS, it is natural to suppose that both conditions are caused by mutations in the same gene. In the interesting family reported by Anna Meadows in 1974, a mother with HH had three children with WT, and one of those has had two children with WT. A tumor from one of the latter cases showed LOH for 11p15 markers, but the lost chromosome 11 markers were the ones derived from the mother, who had had WT, so the very potent gene must be on another chromosome. Yet, a somatic event occurring at 11p15 was evidently critical in tumorigenesis. It may be that HH, and indeed BWS itself, can be caused by two different genes, one at 11p15 and one located elsewhere.

Two large pedigrees segregating numerous cases of WT also showed no linkage with chromosome 11p markers, providing further impetus to a search for another WT gene. The study of some pedigrees indicates heterogeneity in that *WT1* can account for some fraction of hereditary cases. It is noteworthy that penetrance is often very high in hereditary cases, so a third gene might be a major one. It is critical to identify this locus. Since the number of families available for study is small, a major concerted effort should be undertaken to utilize a complete panel of markers in a linkage study.

We see then that much has been learned about the genetics of Wilms' tumor. The *WT1* gene is well-characterized, but accounts for a minority of all cases. The BWS gene and the gene at 11p15 that is critical in many sporadic tumors will probably be cloned in the near future. At least one other gene, still not mapped, accounts for at least some hereditary WTs and perhaps for transforming events in BWS patients. We can look forward to still further excitement at the next Wilms' tumor meeting.